ELECTRON MICROSCOPE STUDIES ON THE NORMAL AND COLCHICINIZED MITOTIC FIGURES OF THE ONION ROOT TIP (ALLIUM CEPA) [1]

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The question of the origin and structure of the achromatic figure in both animal and plant cells is still open to investigation. Various techniques have been brought to bear upon this problem. For a discussion, the monograph of Schrader (1944) should be consulted.

Using the electron microscope, Beams, Evans, Baker and van Breemen (1950), and Beams, Evans, van Breemen and Baker (1950) have studied the structure of the amphiaster in the whitefish blastula and in crayfish testis. In a similar manner, we have observed mitotic figures in root tip cells of *Allium cepa* in an attempt to contribute to knowledge of the structure of the anastral type of spindle, and the origin of the cell plate.

Material and Methods

Onion bulbs were sprouted in tap water at room temperature. Excised root tips were fixed in Randolph's chrome-acetic-formalin mixture for 12 hours, or in weak Flemming's fluid for 15 minutes. One-millimeter segments of the meristematic portion were embedded in either of two filtered paraffin mixtures. At first, a mixture of 70 per cent paraffin (m.p. 70° C.) and 30 per cent bleached beeswax was used. Later, a mixture of 50 per cent Tissuemat (m.p. 62° C.) and 50 per cent beeswax was found to be more satisfactory. In either case, the material was passed through three changes of the mixture, during a total period of 30 minutes. Blocks 2 mm. square were sectioned at approximately 0.5 micron after the method of Beams, Evans, Baker and van Breemen (1950). Ribbons so obtained were spread on distilled water at 45° C. and mounted on grids previously covered with a supporting celloidin film prepared from a 2 per cent solution of celloidin in amyl acetate. The grids were dried over phosphorus pentoxide after sections were mounted. The embedding mixture was then dissolved away in three changes of xylol, and all grids were stored in a desiccator until examined.

In studying the effect of colchicine, two concentrations, 2.5×10^{-4} molar and 2.5×10^{-3} molar, were used.[2] Rapidly growing roots, 0.5 to 1.0 cm. long, were

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[2] The colchicine (U.S.P. XIII alkaloid) used was obtained from the Mallinckrodt Chemical Works.

PLATE I

exposed to these solutions for periods of 30, 45, 60, and 120 minutes. The root tips were then excised, fixed, and processed as described above.

Observations were made using a Model EMU–2B R.C.A. electron microscope equipped with an unbiased electron gun. A magnification scale representing one micron is drawn on each electron micrograph.

Results and Observations

Dividing Cells

1. *The Polar Cap:* Figure 1 is of a longitudinal section through half of a cell in prophase. A polar cap is evident, containing "spindle fibers" which are in contact with the nuclear membrane. In general, these fibers are oriented in the long axis of the cell and extend between the nuclear membrane and a dome-shaped structure which defines the outer margin of the polar cap. This border, in turn, is in contact with the lateral margins of the nuclear membrane.

The fibers are 600 to 800 Å. in diameter in these preparations, and exhibit a beaded structure, possibly due to fixation artifact.

Prior to the breakdown of the nuclear membrane these "spindle fibers" appear entirely extranuclear, and the nucleus contains no distinguishable spindle material.

2. *Metaphase:* Figures 2 and 3 are of longitudinal sections through the equatorial plate. Certain of the fibers seen are undoubtedly associated with the chromosomes, but details of the manner of their attachment are not revealed by this technique.

Chromosomal fibers are composed of smaller units oriented longitudinally to make up the main fiber. The diameter of these smaller units varies somewhat with the fixative used. In material fixed in chrome-acetic-formalin it is 500–800 Å., corresponding to the width of the individual "spindle fibers" of the polar cap. Fixation in weak Flemming's fluid results in fibers of somewhat smaller diameter.

3. *Anaphase:* The separation of homologous chromatids is shown in Figure 4. No interzonal fibers are to be seen.

A later stage is shown in Figure 5. The early cell plate is forming as a series of thickenings in or on the continuous fibers in the equatorial region. The spindle itself appears as a network of interconnecting fibers.

4. *Telophase:* Figure 6 is a later stage in cell plate formation. The cell plate has assumed a more definite structure and has already extended laterally to touch the cell wall on one side. The lateral margins of the spindle in the equatorial region are well defined and oriented. Elsewhere, the spindle material has become swollen and is disappearing. Reconstitution of the daughter nuclei is taking place. Areas of lesser density to the electron beam have appeared in the chromosomes.

Plate I

Figure 1. Prophase, showing polar cap containing "spindle fibers," and part of nucleus with nuclear membrane and chromosomes. Fixed in Randolph's CRAF solution.

Figure 2. Portion of metaphase plate showing chromosomes and spindle fibers. Fixed in Randolph's CRAF solution.

Figure 3. Portion of metaphase plate showing chromosomes and chromosomal fibers. Fixed in weak Flemming's fluid.

Figure 4. Portion of early anaphase showing chromosomes and chromosomal fibers. Fixed in weak Flemming's fluid.

Colchicinized Cells

Figure 7 shows what may be a very early manifestation of the action of colchicine on spindle fibers. The dose used here for a thirty minute exposure was 2.5×10^{-4} molar, considered by Levan (1938) to be in the threshold range for Allium. The chromosomal fibers appear to have lost their compactness by separation of the unit fibers.

Exposure to this same concentration for one hour produces the effect shown in Figure 8. The spindle material is seen to be slightly disoriented and fragmented. The chromosomes themselves appear to have lost much of their smooth contour.

Figure 9 represents a two hour exposure to this same colchicine concentration. The spindle material is swollen and badly fragmented. Much of it has disappeared. Some spindle or cytoplasmic material appears to be adhering to the chromosomes.

Figure 10 results from a 45 minute exposure to a concentration of 2.5×10^{-3} molar colchicine. In general, the effect obtained is comparable to the longer exposures to the more dilute solution.

Discussion

In a study of this kind, the factor of artifact induced by the method of preparation of the material for examination is necessarily present. For this reason, a certain amount of caution must be exercised in interpreting the electron micrographs. In general, however, our results agree with and in some cases extend the cytology as observed with the light microscope.

The fibrous nature of the chromosomal fibers, continuous fibers, and the "spindle fibers" of the polar cap is quite apparent in electron micrographs. The objection that this fibrous structure is a nonsignificant artifact is not considered to be valid today. In this connection, the review of Schrader (1944) should be consulted for details of other work.

With the electron microscope it is possible to demonstrate a compound structure of chromosomal fibers in onion. Beams, Evans, van Breemen and Baker (1950) have similarly shown this to be true in crayfish testis. However, Mottier (1903) had already reported that chromosomal fibers in Lilium were composed of "bundles of fibers" as the result of his observations with the light microscope. He did not observe a similar compound structure of continuous fibers, and we have not seen it in the onion.

In view of the widespread controversy which still exists concerning the production of artifacts in the mitotic spindle by fixation, we have employed two different types of fixatives in this study. The fibers appear of larger diameter when the chrome-acetic-formalin fixation is used than when a very weak Flemming's fluid is employed. However, the nature of the fibers in other respects is not seen to be altered. Preliminary results of a study of the effects of fixation in dilute osmic acid solutions and in osmic acid vapor indicate less swollen (or more contracted) chromosomes, more delicate spindle fibers and cytoplasm than with weak Flemming fixation. The same general character of these structures is, however, retained.

In the course of this work, several examples of a faint and rather ill-defined transverse banding of chromosomal fibers were seen. The phenomenon was ob-

PLATE II

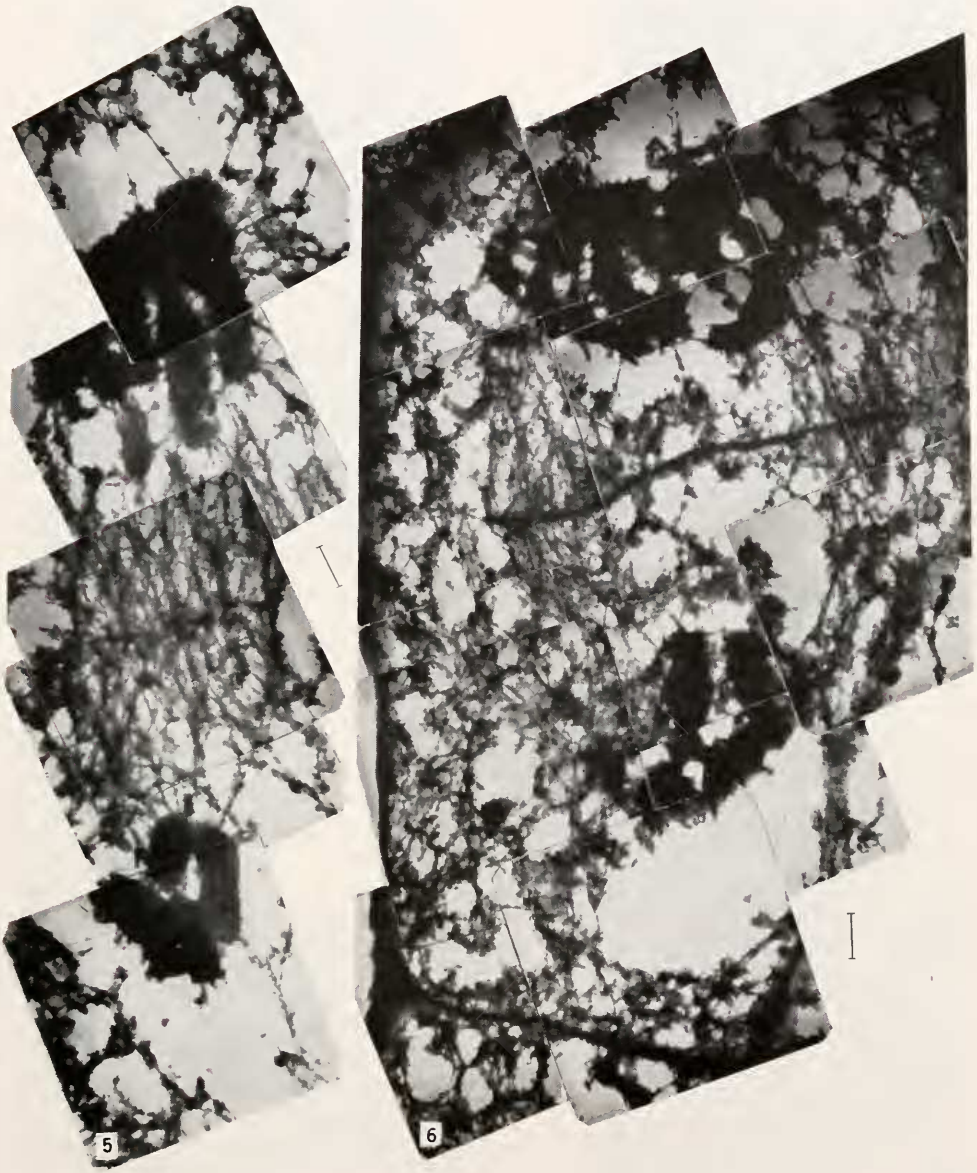

FIGURE 5. Late anaphase, showing spindle, early cell plate and daughter chromosomes. Fixed in Randolph's CRAF solution.

FIGURE 6. Telophase, showing reconstitution of daughter nuclei, phragmoplast, and cell plate extending to cell wall on one side. Fixed in Randolph's CRAF solution.

PLATE III

served in material fixed both in Randolph's and in Flemming's fluids. This banding was noted only in chromosomal fibers and only in metaphase or early anaphase. It does not reproduce well, although it can be seen in the original photographs. The banding is of a much finer nature and of smaller period than that described by Beams, Evans, van Breemen and Baker (1950) and is suggestive of a periodicity in the ultrastructure of the unit fibers. Such a periodicity has been predicted by several workers (see Beams, Evans, van Breemen and Baker 1950), but the phenomenon seen in our preparations cannot be considered anything more than suggestive at this time.

Robyns (1924, 1929) has described in detail the structure of the polar cap and its relation to spindle formation in both living and fixed preparations of onion and similar root tips. For a summary of earlier work, these papers should be consulted. Polar caps are described as clearly differentiated areas in the cytoplasm lying in contact with the nuclear membrane at the poles of the future spindle. In late prophase, these dome-shaped caps may extend as far as the equatorial region of the nucleus. Subsequent to the breakdown of the nuclear membrane, the polar caps enter into the formation of the spindle.

Our preparations show a definite fibrous structure in the polar cap prior to the breakdown of the nuclear membrane. Moreover, the outer margin of the polar cap is clearly seen to be delimited by a membrane-like structure. This membrane appears in electron micrographs to be of much the same structure as the nuclear membrane, and to fuse with it. However, it cannot be said that a definite splitting of the nuclear membrane has occurred in the formation of the polar cap.

The origin of the cell plate in higher plants has been described from fixed material as forming from a series of equatorial swellings in the spindle of cytokinesis (see Timberlake, 1900, for a summary of early work). Robyns (1929) disagrees with this view on the basis of his observations of living material. He concluded that the cell plate makes its appearance in the form of an undulating lamella, and believes that the lamella fragments under conditions of fixation into granules which appear attached to the spindle fibers. Becker (1938) stated that the cell plate first becomes evident as droplets exhibiting Brownian movement. These droplets form from the cytoplasm and eventually coalesce to form the cell plate. Although our preparations are fixed, the earliest indication of cell plate formation which we have observed is a series of thickenings of material continuous with, and of the same appearance as, the spindle fiber substance.

Levan (1938) has observed that the threshold concentration of colchicine which will produce an effect upon mitosis in Allium root tip cells lies in the range of 0.005 to 0.01 per cent with a four hour exposure. Levine (1943) has reported that with the 0.01 per cent concentration, the number of metaphases in Allium root

PLATE III

FIGURE 7. Portion of metaphase plate showing separation of chromosomal fibers resulting from 30 minutes exposure to 2.5×10^{-4} molar colchicine solution. CRAF fixation.

FIGURE 8. Disoriented fibers and eroded chromosomes resulting from 1 hour exposure to 2.5×10^{-4} molar colchicine solution. CRAF fixation.

FIGURE 9. Swollen and fragmented fibers resulting from 2 hours exposure to 2.5×10^{-4} molar colchicine solution. CRAF fixation.

FIGURE 10. Swollen and fragmented fibers resulting from 45 minutes exposure to 2.5×10^{-3} molar colchicine solution. CRAF fixation.

tips increases with increasing time of exposure to reach a maximum at about 24 hours. It is now well known that colchicine brings about the arrest of mitosis by destruction of the spindle. Since our purpose was to study the effects of colchicine upon the spindle fibers themselves, concentrations near the threshold and very short exposure times were chosen.

The very earliest effect which may have occurred was observed to be a spreading apart of the units which make up the compound chromosomal fiber. Longer exposures resulted in a loss of orientation of the spindle fiber material and a change in the normal contour of the chromosomes. Although chromosomes do not dissolve away in colchicine poisoning, Östergren (1944) has reported that colchicine causes a contraction of the chromosomes. Whether or not this has occurred in our preparations is not clear, but it does appear that some cytoplasmic or spindle material has adhered to the chromosomes, or else some of the matrix material has been eroded.

Longer exposure to the more dilute solution, or short exposures to the stronger of the two solutions resulted in swelling of the fibers and apparently a solubilization of their substance, since only fragments remain in the fixed preparations.

Beams and King (1938), using the ultracentrifuge, presented evidence that the effect of colchicine on wheat root tip cells is to lower the viscosity of the cytoplasm and to destroy or inhibit the gelation associated with spindle formation.

We have observed a swelling and fragmentation of spindle material in preparations of normal telophases in connection with the breakdown of the spindle. This swelling and dissolving of spindle material therefore appears to be the visible manifestation of both normal and colchicine-induced spindle breakdown, at least in our fixed preparations.

Summary

1. The electron microscope was used to study normal and colchicinized mitotic figures in onion root tip cells.

2. The polar cap contains "spindle fibers" prior to the breakdown of the nuclear membrane. It is bounded at one margin by the nuclear membrane and on the other by a membrane similar to and apparently continuous with the nuclear membrane. No spindle material is distinguishable inside the nucleus prior to the breakdown of the nuclear membrane.

3. Chromosomal fibers are composed of several smaller unit fibers oriented in a longitudinal fashion along the main axis of the fiber. This compound structure was not observed in continuous fibers.

4. Spindle fibers differ somewhat in diameter with different fixation. Flemming's fluid fixation results in a slightly smaller fiber diameter than that obtained with fixation in Randolph's CRAF mixture. The general character of the fibers in both preparations is essentially the same.

5. The origin of the cell plate is briefly discussed in the light of the work of early investigators. Electron micrographs show an origin by thickenings in the equatorial region of the spindle.

6. The effect of short exposures to near-threshold concentrations of colchicine is a progressive swelling and solubilization of fiber material.

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